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#10 (NE)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: **Covacci *et al.***

Serial No.: **09/360,685** Group Art Unit: **1645**

Filed: **July 26, 1999** Examiner: **P. Bui**

For: **HELICOBACTER PYLORI CAI ANTIGEN PROTEINS USEFUL FOR VACCINES AND DIAGNOSTICS**

I, **Robin S. Quartin**, Registration No. **45,028** certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.
On Aug 3, 2000

Robin S. Quartin Reg. No. **45,028**

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

I, Giuseppe Del Giudice, do hereby declare as follows:

1. I am a Research Director employed by Chiron SpA, in Siena, Italy.

2. I am a medical doctor (M.D.) with 15 years of experience in vaccine development.

3. I have read U.S. application serial number 09/360,685, filed July 26, 1999 entitled "*Helicobacter pylori* CAI antigen proteins useful for vaccines and diagnostics" ("'685 application"). While I am not an inventor of the subject matter of the '685 application, I am quite familiar with the invention and the technology at issue. The '685 application claims priority to PCT/EP93/00158 (filed January 25, 1993) and PCT/EP93/00472 (filed March 2, 1993), which two PCT applications claimed priority benefit of Italian application Serial No. FI92A000052 (filed March 2, 1992).

4. The invention provides polypeptides of the *Helicobacter pylori* cytotoxin associated immunodominant antigen (CagA)[1], for use, among others, in vaccines. The CagA polypeptides should induce an immune response, should not be toxic, and preferably, should protect against subsequent challenge by the pathogen.

5. I have read the Official Action dated February 14, 2000 ("Action").

6. In the Action, the Examiner has rejected claims 42 - 65 as allegedly being indefinite because of the use of the term "substantially," which the Examiner asserts is a relative term lacking comparative basis. I respectfully disagree.

7. The term "substantially" is used in the amended and original claims in conjunction with terms relating to toxicity in the phrase "exhibits no functional contribution to toxicity, or a substantially reduced functional contribution to toxicity." This phrase is used to describe the characteristics of the claimed *H. pylori* CagA polypeptides, and the *H. pylori* CagA and heat shock protein polypeptide components of the claimed vaccines of the invention. The term "substantially," as it is used in the

[1] In the specification of the '685 application, *H. pylori* cytotoxin associated immunodominant antigen is referred to as "CAI." However, the current terminology used for this protein is the "cytotoxin-associated gene A" or "CagA" antigen.

'685 application, would have been clearly understood by those of skill in the art, and is understood to mean that such *H. pylori* polypeptides do not exhibit statistically significant cytotoxic effects and, thus, would be acceptable for use in human vaccines. Cytotoxicity can be routinely assessed in a variety of assays known to those of skill in the art, such as *in vitro* vacuolation of cells or cell lines, *e.g.* HeLa cells, and *in vivo* administration of *H. pylori* polypeptides to mice to analyze gastric tissue damage.

8. In the Action, the Examiner rejected claims 42 - 65, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with the claims. The Examiner asserted that undue experimentation would have been required to determine which polypeptides/proteins are effective for use as vaccines and which amino acid sequences of CagA protein would "exhibit substantially no contribution to toxicity" or would be effective as components in a "prophylactic or therapeutic vaccine," at the time of filing. I respectfully disagree.

9. It is routine to determine, and was routine to determine as of March 2, 1992, which ten or fifteen amino acids of *H. pylori* CagA would exhibit no functional contribution to toxicity, or a substantially reduced functional contribution to toxicity, immunogenic, and functional as a vaccine.

10. Protein fragments for testing are easily generated through such means as recombinant expression techniques, using the sequences disclosed in the '685 application. Thus, it would have been routine, as of March 2, 1992, to generate fragments of *H. pylori* polypeptides for determination of which ten or fifteen amino acids of CagA or heat shock protein would exhibit no functional contribution to toxicity, or a substantially reduced functional contribution to toxicity, be immunogenic, and be functional as a vaccine.

11. Regarding toxicity, many *in vitro* and *in vivo* assays are and were known to those of skill in the art, which can be used for the routine determination of those fragments of CagA and heat shock protein that are cytotoxic and those that are not. Such testing is standard in the development of a vaccine, where all components are assessed for cytotoxicity, as vaccines having cytotoxicity would be unacceptable for use in humans. Toxicity testing assays have been used by those of skill in the art to make such determinations since long before the filing date of the '685 application, and priority applications. *See* Goodman & Gilman, eds., The Pharmacological Basis of Therapeutics, 5th Ed., Macmillan Publishing, New York, 1975.

12. Exhibit A is a copy of Reyrat *et al.*, 1999, *Mol. Microbiol* 34:197-204, which is a review of the structure and activity of *H. pylori* cytotoxin (VacA) protein[2]. VacA was first described to induce vacuolation (the formation of large vacuoles) of mammalian cells *in vitro*, in 1988 (*see* page 199, column 2 of Exhibit A). Fig. 1A of Exhibit A shows a microscopic view of the vacuolating activity of purified VacA protein on HeLa cells. Thus, at the time of filing, those skilled in the art could have used an *in vitro* vacuolation assay on mammalian cells to routinely distinguish toxic and non-toxic *H. pylori* polypeptides.

13. Animal models used for the study of *H. pylori* infection were known prior to March 2, 1992. Such animal models of *H. pylori* infection include the gnotobiotic piglet (Krakowka *et al.*, 1987, Infect. Immun. 55:2789-2796) (Exhibit B) and the gnotobiotic dog (Radin *et al.*, 1990, Infect. Immun. 58:2606-2612) (Exhibit C). Animal models present convenient *in vivo* assay systems for routinely distinguishing non-toxic VacA protein, or fragments thereof, from those which exhibit toxicity. For example, Telford *et al.*, 1994, *J. Exp. Med.* 179:1653-1658 (Exhibit D), describes an

[2]In the specification of the '685 application, *H. pylori* cytotoxin is referred to as "CT." However, current terminology for the "vacuolating" cytotoxin protein of *H. pylori* is "VacA."

in vivo mouse model of *H. pylori*-induced gastric ulceration used to show that the VacA protein is responsible for the epithelial erosion seen in *H. pylori* infection. Sonicated, VacA-producing *H. pylori* cells were shown to induce erosive lesions of the gastric mucosa, when orally administered to mice (Figure 1 (b and c)). Administration of purified VacA to mice also resulted in extensive tissue damage and mucosal erosion (Figure 1 (d, e, and f)). Thus, *H. pylori* infection models can be used, and could have been used at the time of filing, for routinely distinguishing toxic and non-toxic *H. pylori* CagA and heat shock polypeptides.

14. Regarding immunogenicity, one of skill in the art could have employed classical immunological assays to screen for antibody production in response to immunizations with fragments of *H. pylori* cytotoxin protein. These include, for example, 1) enzyme-linked immunosorbent assay (ELISA), 2) proliferation assays of cells from lymphoid organs, and 3) evaluation of the number of cells producing antibodies to a given antigen. Detailed protocols for these standard assays can be found in any manual on immunology. The Handbook of Experimental Immunology, Weir & Blackwell (eds.), 1986, which is cited at page 5, lines 22 - 24 of the specification, is a good example of such a manual, available to those of skill in the art at the time of filing of the application. Current Protocols in Immunology, John Wiley & Sons, New York, NY, which has been published since 1991, is another example of such a manual available to those of skill in the art. Thus, it would have been routine to determine which fragments of CAI or heat shock protein would generate an immune response, at the time of filing of the '685 application.

15. Regarding vaccine function, at page 15, lines 14 - 17, the specification of the '934 application defines a vaccine as "capable of eliciting protection against *H. pylori*." Furthermore, the vaccines of the invention can be prophylactic, therapeutic, or both (*see* page 38, line 39 - page 40, line 2, of the '934 application). Demonstration of a prophylactic or therapeutic effect of a protein, or polypeptide fragment of a protein, could have been carried out using routine functional experiments

and assays. Functional experiments include the administration of a candidate vaccine to animals susceptible to *H. pylori* infection, either before challenge with the pathogen (prophylaxis determination) or after infection has taken place (treatment determination). Animal models of disease provide convenient environments for such vaccine testing. *See* ¶ 13 above.

16. Exhibit E is a copy of Nedrud, 1999, *FEMS Immunol. Med. Microbiol.* 24:243-50, which is a review of animal models of *H. pylori* infection that have been established, including the pig, dog, gerbil, monkey, and ferret. Such models have been used since 1987 to examine infection-related disease processes and evaluate vaccines, and they can be used routinely to determine the effectiveness of *H. pylori* proteins and polypeptide fragments as vaccines against the infection.

17. Exhibit F is a copy of Ghiara *et al.*, 1997, *Infect. Immun.* 65:4996-5002, of which I am a co-author, and which shows that therapeutic vaccination with full-length recombinant *H. pylori* proteins, including VacA, can eradicate chronic *H. pylori* infection in a mouse model, and protect against subsequent challenge. Figure 3 of Exhibit F presents the results of therapeutic vaccination. Vaccination with full-length recombinant VacA protein (indicated as "Tox100") resolved the infection in about 92% of the mice. Full-length recombinant CagA protein yielded a 70% eradication of infection rate. Furthermore, once therapeutically treated, the mice are also protected from further challenge with *H. pylori*. Figure 5 presents the results of a study of reinfection rate, and shows that therapeutic vaccination with the recombinant VacA protein protected 70% of the mice from reinfection with *H. pylori*.

18. In the Action, the Examiner also asserted that a mucosal adjuvant is required for effective *H. pylori* component vaccines. I respectfully disagree with this characterization of the state of the art. Exhibit G is a copy of published PCT application PCT/IB99/00851, of which I am a co-inventor, and which teaches that mucosal delivery and mucosal adjuvants are <u>not</u> required for

effective *H. pylori* component vaccines. This PCT application presents the results of intramuscular immunization studies with *H. pylori* component vaccines, in a dog model. Page 21 presents the protocol for immunization. Full-length recombinant *H. pylori* proteins -- VacA, CagA, and neutrophil activating protein (NAP) -- were used as vaccine components for intramuscular immunizations. The adjuvant was aluminum hydroxide, *i.e.*, not a mucosal adjuvant

19. Exhibit G demonstrates that the intramuscular immunizations induced high serum titers of antigen-specific antibodies to each of the *H. pylori* component proteins in the vaccine (*see* Figure 5A (VacA), Figure 5B (CagA), and Figure 5C (NAP)). Furthermore, intramuscular immunization was effective to protect all of the dogs from challenge with *H. pylori*. No symptoms of *H. pylori* infection were evident in the intramuscularly vaccinated dogs (page 12, lines 24 - 25). At 10 and at 42 days post-challenge, the intramuscularly vaccinated dogs' antral biopsies and gastric lavages were negative for urease[3] activity (page 12, lines 26 - 29, and page 14, Table 2). Furthermore, at 42 days post-challenge, intramuscularly vaccinated dogs had normal mucosa, without the signs of hyperemia or edema seen in the *H. pylori*-infected control animals (page 14, lines 4 - 10, and Figures 1A and 1B).

20. In the Action, the Examiner also rejected the claims as anticipated by Cover *et al.* (1990). I respectfully disagree.

21. The claims, as amended, are directed to (1) purified *H. pylori* CAI (CagA) polypeptides, (2) *H. pylori* CAI (CagA) polypeptides capable of inducing the production of anti-*H. pylori* antibodies, and exhibiting no functional contribution to toxicity, or a substantially reduced functional

[3]Urease is another *H. pylori* protein (*see* '685 application page 2, lines 8 - 14). A urease activity assay (*see* '685 application page 46, lines 14 - 16) is a means of detecting the presence of *H. pylori* infection, both in the experimental and in the clinical setting.

contribution to toxicity, and (3) vaccines comprising *H. pylori* CAI (CagA) polypeptides capable of inducing the production of anti-*H. pylori* antibodies, and exhibiting no functional contribution to toxicity, or a substantially reduced functional contribution to toxicity.

22. Cover *et al.* (1990) discloses a 128 kDa *H. pylori* protein that is recognized by antibodies in human sera in an immunoblot. Cover *et al.* (1990) did not purify this 128 kDa *H. pylori* protein. Cover *et al.* (1990) does not teach or disclose purified polypeptides of the 128 kDa protein that exhibit no functional contribution to toxicity, or a substantially reduced functional contribution to toxicity. Cover *et al.* (1990) does not disclose a vaccine.

23. Cover *et al.* (1990), is a study of the anti-*H. pylori* specificities of human sera. Among other findings, Cover *et al.* (1990) showed that sera from patients infected with *H. pylori* (and from 6% of uninfected patients) contain antibodies that recognize a 128 kDa *H. pylori* protein, associated with strains of *H. pylori* having vacuolating activity [4]. This 128 kDa protein is generally accepted today to be the CagA protein of *H. pylori*. Cover *et al.* (1990) did not purify the 128 kDa protein. Cover *et al.* (1990) were unable to visualize the 128 kDa protein on a silver stain gel of concentrated culture supernatants from H. pylori cultures (page 606, Figure 2). Cover *et al.* (1990) demonstrated recognition, by human sera, of the 128 kDa protein in immunoblots of concentrated culture supernatant from a toxigenic *H. pylori* strain (page 607, Figure 4). Those of skill in the art know that immunoblots only reveal the presence of a protein in a heterogeneous mixture of proteins. Without further processing steps, an immunoblotting signal identifying a protein's position on a filter does not yield a purified protein. Hundreds of contaminating proteins and other molecules will be present, in proximity to, and in contact with, the protein of interest. These are contaminants that

[4] The terms vacuolating activity or vacuolizing activity interchangeably describe the ability to induce the formation of large vacuoles in cells.

were present in the gel and transferred to the filter, or that were added to the filter during the blocking and probing steps. Since Cover *et al.* (1990) did not purify the 128 kDa protein of *H. pylori*, Cover *et al.* (1990) clearly did not purify *H. pylori* CagA protein.

24. Cover *et al.* (1990) did not investigate nor did they present any data on a toxic or non-toxic activity of the 128 kDa protein. The reference reports the presence of the 128 kDa protein (as detected by human serum) in all *H. pylori* culture supernatants having vacuolating activity and in 64% of the culture supernatants without vacuolating activity, and refers to the 128 kDa protein as "a potential marker for vacuolizing cytotoxin activity" (page 607).

25. Cover *et al.* (1990) does not describe or suggest a vaccine, but only describes the generation of rabbit antisera to *H. pylori* components, without the demonstration of a prophylactic or therapeutic effect. Furthermore, Cover *et al.* (1990) describes the generation of an immune response in rabbits to some *H. pylori* proteins, but not to the 128 kDa protein. A rabbit antiserum, raised against supernatant from a toxigenic *H. pylori* strain, did not reveal a 128 kDa protein in immunoblots (page 606, Figure 3, and page 609, column 1).

26. In the Action, the Examiner also rejected the claims as obvious over Cover *et al.* (1990). I respectfully disagree.

27. Cover *et al.* (1990) describes three types of *H. pylori* culture preparations for use in immunization of rabbits (page 604, column 2). The first type of preparation was concentrated *H. pylori* culture supernatant, the second was formalinized, whole *H. pylori* cells, and the third was supernatant of French pressure cell-treated whole *H. pylori* cells. The preparations as injected into the rabbits would not be considered by those of skill in the art to inherently contain

"pharmaceutically acceptable carriers," such as those used in the methods of preparing vaccines intended for human use.

28. I declare that all statements made herein are of my own knowledge true and statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Giuseppe Del Giudice, M.D. Date